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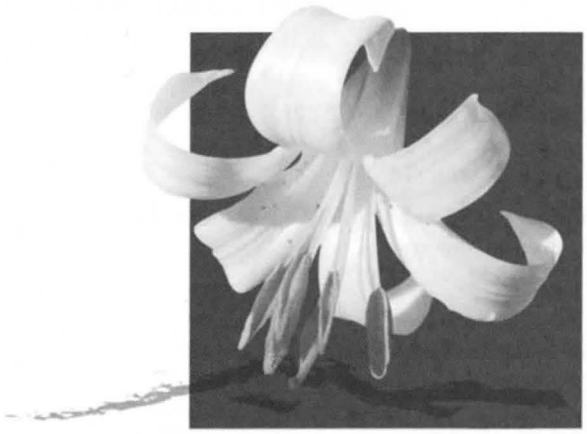

MONOCOTS

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SYSTEMATIC FLORAL ANATOMY OF PONTEDERIACEAE

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ABSTRACT

Twenty species of Pontederiaceae and six species of the outgroup families Haemodoraceae and Philydraceae were investigated with regard to floral anatomy, using standard histological methods and graphic reconstructions. Variation is described in several features, including functional carpel number, the distribution of floral aerenchyma, the presence of unusual floral epithelial cells, the presence, type, and distribution of tannin cells, crystal type and distribution, aspects of placentation, ovule number, number of ovule rows per carpel, and septal nectary presence. In order to better assess character homology, one of these features, placentation, was divided into three discrete characters: septal fusion, placenta position, and placenta evagination. Several of the floral characters were optimized onto a most parsimonious cladogram from a relatively recent study using combined chloroplast DNA data. Associations of character states with specific clades were observed in functional carpel number, aerenchyma distribution, epithelial cell presence, tannin cell distribution, septal nectary presence, and the three redefined aspects of placentation: septal fusion, placenta position, and placenta evagination. These floral data will be valuable in further elucidating relationships in Pontederiaceae in future studies of the group.

Key words: anatomy, floral, flower, phylogeny, placentation, Pontederiaceae.

INTRODUCTION

Pontederiaceae Kunth are a pantropical family of herbaceous, annual or perennial, freshwater aquatic monocotyledonous plants (Hutchinson 1973; Cronquist 1981; Takhtajan 1997). A recent treatment of the family by Cook (1998) listed nine genera and approximately 33 species, although other treatments unite these nine genera into as few as four. Members of the family occur mainly in the neotropics, with a few species ranging as far north as southeastern Canada and as far south as southern Argentina. Other species are found in South Africa, Madagascar, Southeast Asia, and Australia. *Eichhornia crassipes,* the water hyacinth, is probably the most well-known member of the family, since it was introduced to many tropical and subtropical areas, perhaps originally as an aquatic ornamental. It is considered one of the world's most troublesome aquatic weeds (Gopal 1987; cited in Cook 1998).

In recent phylogenetic analyses, Pontederiaceae has been consistently allied in various combinations to Commelinaceae, Haemodoraceae, and Philydraceae (Duval et al. 1993; Chase et al. 2000). Morphological studies of monocots have also supported the close relationships among Haemodoraceae, Philydraceae, and Pontederiaceae, but the compiled evidence has been ambiguous about the exact sister group relationships. Simpson (1987) hypothesized that Pontederiaceae shares a most recent common ancestor with Haemodoraceae by inferring homology in pollen exine sculpturing; both taxa share a one- to two-layered nontectate-columellate exine architecture, and some a verrucate sculpturing (see also Eberhardt 1996). In addition, Simpson (1993) points out that some members of both Haemodoraceae and Pontederiaceae possess septal nectaries while all members of Philydraceae do not; however, septal nectaries are likely an ancestral character for the monocots. Tillich (1995), in an analysis of monocot seedling morphology, noted that both Philydraceae and Pontederiaceae possess a bifacial, linear cotyledon, a potentially unique feature in angiosperms. Haemodoraceae, on the other hand, possess a variety of cotyledon forms, all of which are unifacial.

The intrafamilial systematics of Pontederiaceae has varied somewhat (see Eckenwalder and Barrett 1986; Graham and Barrett 1995). The family was divided by Schwartz (1930) into three tribes: Eichhornieae, Heteranthereae, and Pontederieae. In his treatment of Pontederiaceae, Cook (1998) cites the three tribes of Schwartz. However, Cook argues that *Monochoria* should be placed in Eichhornieae, not Heteranthereae (as placed by Schwartz), based on morphological features.

According to Cook (1998), tribe Pontederieae includes two genera, *Pontederia* L. (3–6 spp.) and *Reussia* Endl. (2 spp.), the latter sometimes recognized as a subgenus of *Pontederia* (Lowden 1973; Eckenwalder and Barrett 1986; Rosatti 1987; Graham and Barrett 1995). Members of Pontederieae have a zygomorphic, tubular perianth, and six stamens. The tribe is distinctive in having a uniovulate ovary with one functional carpel, the whole developing into a one-seeded fruit surrounded by a withered, accrescent, hardened perianth tube, a fruit type termed an "anthocarp." *Reussia* is distinguished from *Pontederia* by the presence of a spiny anthocarp. Pontederieae is perhaps the most well-supported intrafamilial grouping because of this distinctive ovary and fruit morphology. Members of tribe Eichhornieae, which includes *Eichhornia* Kunth (7 or 8 spp.) and *Monochoria* C. Presl (8 spp.), have a zygomorphic, tubular perianth, six stamens, and a multi-seeded, trilocular capsule. Although *Monochoria* resembles *Eichhornia* and *Pontederia* in having a geniculate infructescence, *Monochoria* is unique in the family in having a perianth fused only at the extreme base. Eichhornieae and Pontederieae are possibly linked via the zy-

gomorphic perianth and a geniculate infructescence (Rosatti 1987). Eckenwalder and Barrett (1986), as well as Graham and Barrett (1995), noted that these taxa constitute a group of mainly tristylous species, suggesting that tristyly is ancestral for this complex. With the exception of one species (*Eichhornia natans* Solms, found in South Africa), these two tribes are native to the New World.

Tribe Heteranthereae is characterized by the presence of one to three dimorphic stamens with basifixed anthers, often cleistogamous, more or less actinomorphic flowers, and a largely enantiostylous floral syndrome (Eckenwalder and Barrett 1986; Graham and Barrett 1995). Within Heteranthereae, Cook chooses to recognize *Heteranthera* Ruiz & Pav. s.s., plus the four segregate genera of *Eurystemon* Alexander, *Hydrothrix* Hook f., *Scholleropsis* H. Perrier, and *Zosterella* Small, for a total of nine genera and approximately 33 species in the family. Following the convention of Graham et al. (1998), we merge both *Eurystemon* and *Zosterella* into *Heteranthera* in this study, but treat *Hydrothrix* and *Scholleropsis* as distinct genera.

A recent analysis of Pontederiaceae (Graham et al. 1998; see also Kohn et al. 1996) examined phylogenetic relationships using both chloroplast DNA data and morphological data, noting a significant discrepancy between the two. Although tree rooting was problematic, results of this study indicated monophyletic tribes Pontederieae and Heteranthereae (excluding *Monochoria,* as in Cook 1998), but with tribe Eichhornieae forming a grade at the base of Pontederieae. (See Discussion, below.)

Because of the uncertainty of intrafamilial relationships in Pontederiaceae, additional characters are needed to assess these relationships more robustly. This is particularly important in tracing the evolution of adaptively significant features, e.g., those involving reproductive mechanisms such as heterostyly (Graham and Barrett 1995; Barrett and Graham 1997). The purpose of this study is to identify floral anatomical characters and to optimize character state changes, using recently generated cladistic hypotheses (Graham et al. 1998). The correlation of these features with putative monophyletic groups within the family is explored, with emphasis on their possible adaptive significance. This study expands that of Burton and Simpson (1996) and supplements a recent study by Strange et al. (2004). (See Discussion, below.)

MATERIALS AND METHODS

Mature buds or recently opened flowers were preserved in the field or greenhouse in FAA (formalin-acetic acid-alcohol), embedded in Paraplast, and sectioned serially, both transversely and longitudinally. Section thickness was generally 10 μm, but occasionally ranged from 8–15 μm depending on the flower size. Sections were stained with safranin, fast-green, and hematoxylin according to standard procedures (Johansen 1940; Sass 1958; Berlyn and Miksche 1976). Light microscope images were photographed using a Nikon Microphot-FX microscope with a Nikon Coolpix 990 digital camera (Fig. 1–41).

Twenty species in Pontederiaceae, two of Haemodoraceae, and two of Philydraceae were examined. Voucher information is presented in Table 1.

Sections were studied with regard to presence, type, and distribution of crystals, tannin cells, aerenchyma, septal nectaries, and specialized anatomical features of cells and tissues (see Table 2). Descriptions of floral anatomical features were made for each species examined. In order to better visualize the spatial distribution of major floral components, we prepared a diagram showing the relative extent and position of the locular cavity, septal tissue (illustrating fused and unfused regions), ovule attachment, and septal nectaries of each species examined (Fig. 42). For three species a detailed graphical representation of a flower longitudinal section was prepared from sequential cross sections according to the procedure outlined in Simpson, 1998 (Fig. 43–45). A diagram was prepared illustrating various placentation features (Fig. 46–50). Finally, in order to assess the evolutionary history of examined traits, several floral anatomical characters were optimized, onto one of the cladograms of Graham et al. (1998) using MacClade (Maddison and Maddison 1992; Fig. 51–58). Note that we chose one of the most parsimonious cladograms, derived from combined chloroplast *ndh*F, *rbc*L, and restriction-site variation data of Graham et al., 1998 (their Fig. 2, right side, with their undescribed "*Eichhornia* sp." omitted). We have also added two outgroups (Haemodoraceae and Philydraceae) on these phylogenies in order to better assess relative ancestral character states.

RESULTS

Eichhornia crassipes

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous; Fig. 1–3). The septa are barely fused at the apex of the ovary, with a commissure not clearly present. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in two to four rows per flange. The region of ovule attachment extends continuously from near the base to the apex of locule (Fig. 42). Septal nectaries are present, in the basal region of the ovary. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, perianth, ovary wall, placentae, stamen filaments, and connective, especially concentrated in placentae. Tannin cells are mainly homogeneous, with some granular tannin cells; both are distributed in tissues of the receptacle, perianth, ovary wall, septa (very sparsely), placentae, and style. (See Fig. 1–4; Table 2).

Eichhornia diversifolia

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous); commissures do not occur at the junction of the septa. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in two rows per flange (becoming one row near the apex). The region of ovule attachment is continuous from near the base to the apex of the locule (Fig. 42). Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed sparsely in tissues of the receptacle, perianth, and ovary wall. Tannin cells are homogeneous and fibrillar and are distributed in tissues of the receptacle, perianth, ovary wall, and style, sparsely in septa. (See Fig. 8; Table 2).

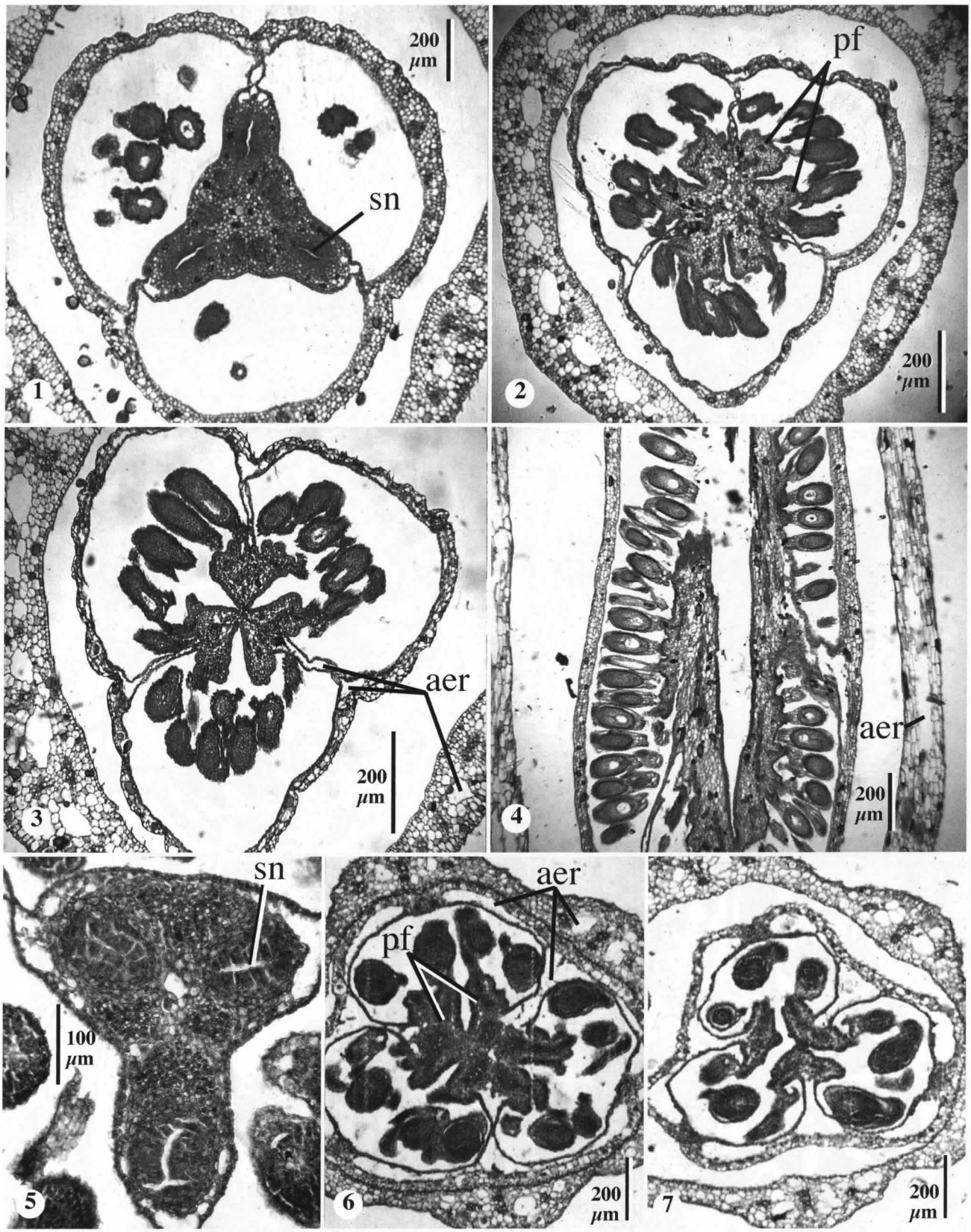

Fig. 1–7.—1–4. *Eichhornia crassipes.*—1–3. Serial cross sections.—1. Base of ovary, showing septal nectaries (sn).—2. Middle region of ovary, showing two placental flanges (pf) and 2–4 rows of ovules per flange.—3. Top region of ovary, showing barely fused septa. Note aerenchyma (aer) in perianth, ovary wall, and septa.—4. Flower longitudinal section. Note pleurotrous ovules and aerenchyma (aer).—5–7. *E. paniculata,* cross sections.—5. Septal nectaries (sn) near base of ovary.—6. Midregion of ovary, showing aerenchyma (aer), synseptaly, and flanged placentae (pf) with two rows per flange.—7. Ovary apex, showing aposeptaly.

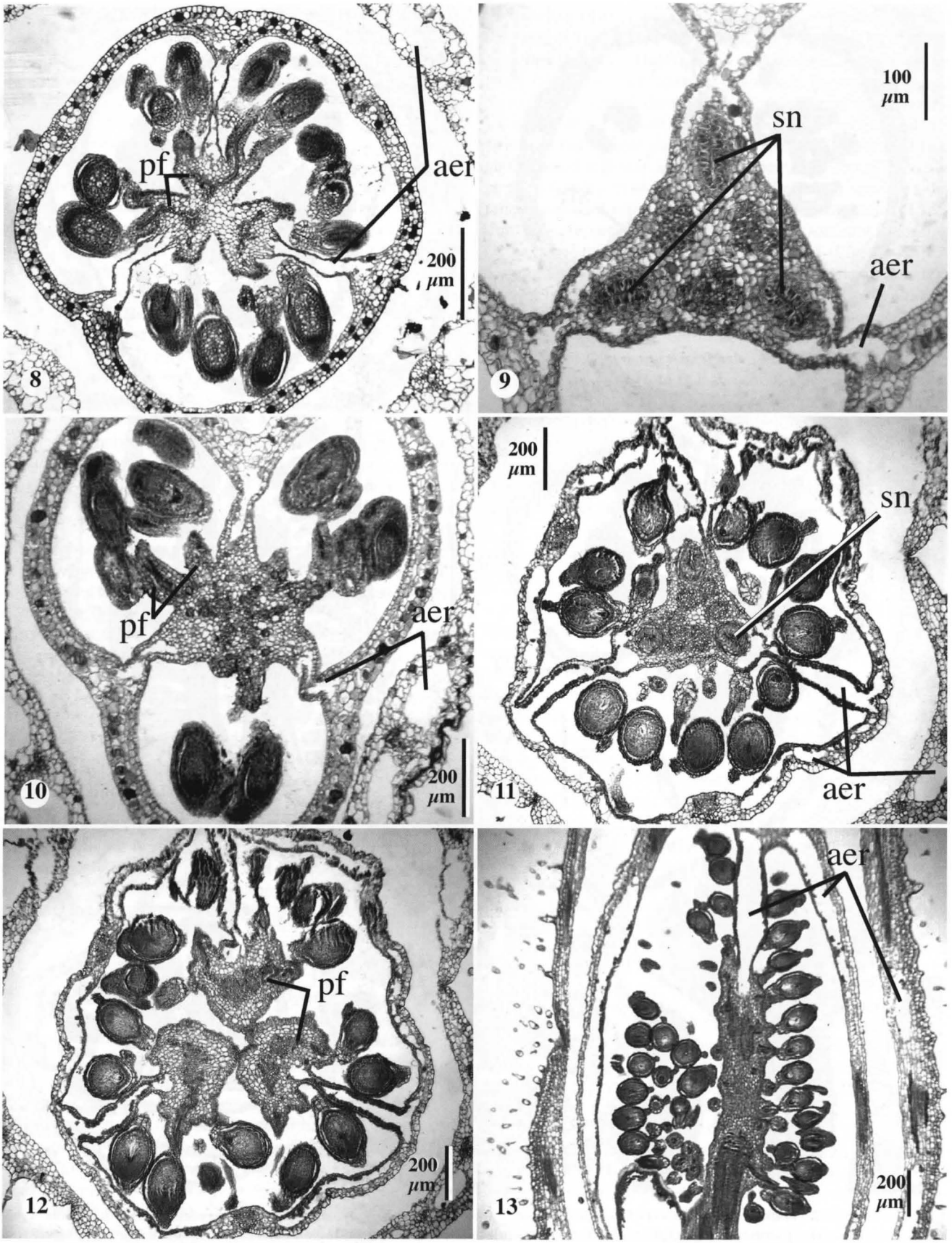

Fig. 8–13.—8. *Eichhornia diversifolia,* cross section. Note two placental flanges (pf) per carpel and aerenchyma (aer).—9–10. *Eichhornia heterosperma.*—9. Ovary base, with septal nectaries (sn) and aerenchyma (aer).—10. Midregion of ovary, showing placental flanges (pf) and aerenchyma (aer).—11–13. *E. meyeri.*—11–12.—Serial cross sections.—11. Base of ovary with septal nectaries (sn) and aerenchyma (aer).—12. Apex of ovary, showing placental flanges (pf).—13. Longitudinal section, showing aerenchyma (aer) in perianth, ovary wall, and septal regions.

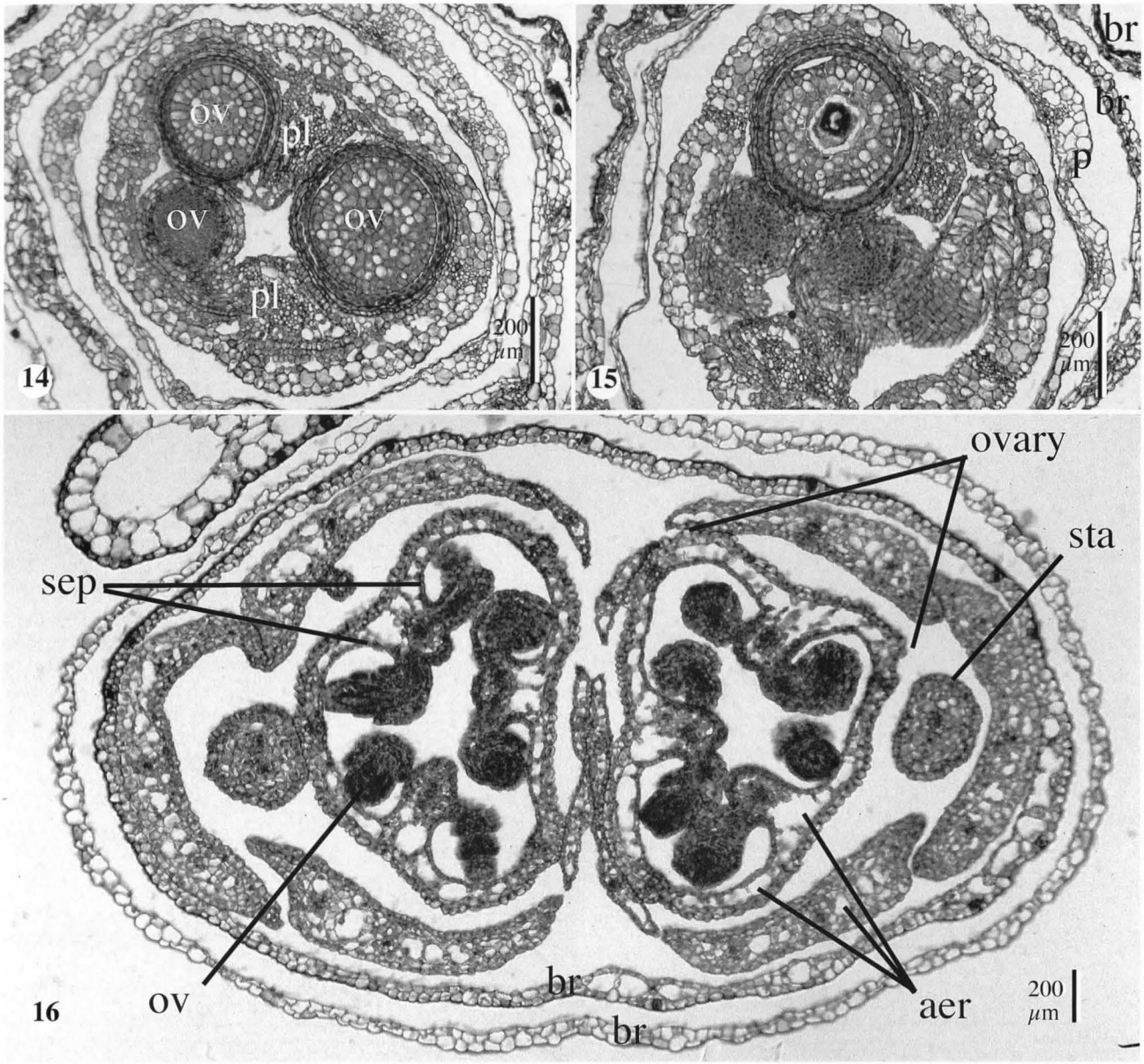

Fig. 14–16.—14–15. *Heteranthera dubia,* flower cross sections.—14. Lower ovary region, showing placentae (pl) and ovules (ov), surrounded by ovary wall and perianth.—15. Upper ovary region. Note bracts (br) surrounding perianth (p).—16. *Hydrothrix gardneri,* flower cross section. Note paired flowers, showing septa (sep), ovules (ov), ovary wall, stamen filaments (sta), and surrounding bracts (br). Note aerenchyma (aer) in several tissues.

Eichhornia heterosperma

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous). However, commissures at the junction of the septa are present near the apex of the ovary. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in one row per flange. The region of ovule attachment extends continuously from near the base to the apex of the locule (Fig. 42). Septal nectaries are present, in the basal region of the ovary. Aerenchyma is present in tissues of the receptacle, perianth, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the perianth, ovary wall, and placentae. Tannin cells are fibrillar (rarely homogeneous) and are distributed in tissues of the receptacle and style, sparsely in the perianth, ovary wall, and septa. (See Fig. 9, 10; Table 2).

Eichhornia meyeri

Placentation is axile-parietal, with three carpels and three locules at ovary base, one locule at the apex. The septa are fused from the basal to the middle region of the locule, unfused at the apex (hemiseptalous). Commissures are not present at the junction of the septa. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in two to three rows per flange. The region of ovule attachment is continuous from near the base

Table 1. Voucher information.

Taxon	Voucher
PONTEDERIACEAE	
Eichhornia crassipes (Mart.) Solms	*Barrett 93-15*
E. diversifolia Urb.	*Barrett 93-14*
E. heterosperma Alexander	*Horn 4545*
E. meyeri A. G. Schulz	*Barrett 93-2*
E. paniculata Solms	*Horn 4547*
Heteranthera dubia Morong [*Zosterella dubia* Small]	*Barrett 93-11*
H. mexicana S. Watson [*Eurystemon mexicanum* (S. Watson) Alexander]	*Graham 7-7-95* *Horn 2215*
H. multiflora (Grisebach) C. N. Horn	*Horn 549*
H. reniformis Ruíz & Pav.	*Simpson 4VIII82A*
H. rotundifolia Grisebach	*Horn 512*
H. seubertiana Solms	*Barrett 93-12*
H. zosterifolia Mart.	*Barrett 93-13*
Hydrothrix gardneri Hook f.	*Barrett 93-9; Horn 532*
Monochoria hastata (L.) Solms	*Barrett 93-5*
M. korsakowii Regel & Maack	*Barrett 93-4*
M. vaginalis (Burm. f.) C. Presl ex Kunth	*Oswald & Ahart 4854*
Pontederia cordata L. var. *cordata*	*Simpson 30VI86A*
P. sagittata C. Presl.	*Barrett 93-7*
Reussia subovata Solms	*Graham 5-31-95*
Scholleropsis lutea H. Perrier	*Mabberley 741*
HAEMODORACEAE	
Haemodorum spicatum R. Br.	*Simpson 16IX81C*
Tribonanthes variabilis Lindl.	*Simpson 8IX81A*
PHILYDRACEAE	
Helmholtzia acorifolia F. Muell.	*Simpson 81-16A*
Philydrum lanuginosum Gaertn.	*Hamann 959*

to the apex of the locule (Fig. 42). Septal nectaries are present, in the basal region of the ovary. Aerenchyma is present in tissues of receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, perianth, and ovary wall. Tannin cells are granular and very sparse in receptacle, placental, and stylar tissue, but otherwise absent. (See Fig. 11–13; Table 2).

Eichhornia paniculata

Placentation is axile-parietal, with three carpels and three locules at ovary base, one locule at the apex. The septa are fused from the base to the middle region of the locule (hemiseptalous). Commissures are present at the junction of the fused septa near the apex. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in two rows per flange (becoming one row near apex). The region of ovule attachment is continuous from near the base to the apex of the locule (Fig. 42). Septal nectaries are present, in the basal region of the ovary (Fig. 42, 43). Aerenchyma is present in tissues of receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the perianth, ovary wall, septa, placentae, and funiculus. Tannin cells are granular and sparse in receptacle tissue, but otherwise absent. (See Fig. 5–7, 43; Table 2).

Heteranthera dubia [Zosterella dubia]

Placentation is parietal, with two carpels and one locule. The septa are unfused throughout (aposeptalous). The placentae consist of two slightly evaginated flanges. Ovules are epitropous and hypotropous, numerous, in one row per flange. The region of ovule attachment extends continuously from the base to the apex of the locule (Fig. 42). Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa/placentae. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle and perianth. Tannin cells are homogeneous and granular and are distributed in tissues of the receptacle, ovary wall, septa, placentae, style, and androecium, very sparsely in the perianth. (See Fig. 14, 15; Table 2).

Heteranthera mexicana [Eurystemon mexicanum]

Placentation is parietal, with three carpels and one locule. The septa barely touch, with commissures at their junction and a large intercellular space at the center of the junction region. The placentae consist of two slightly evaginated flanges. Ovules are mostly pleurotropous, numerous, in two rows per flange. The region of ovule attachment extends from the base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, perianth, ovary wall, and septa. Tannin cells are absent. (See Fig. 17, 18; Table 2).

Heteranthera multiflora

Placentation is parietal, with three carpels and one locule. The septa are unfused throughout (aposeptalous). Commissures at the junction of the septa are absent (septa not fused). The placentae consist of two evaginated flanges. Ovules are mostly pleurotropous, numerous, in two to three rows per flange. The region of ovule attachment is continuous from the base to the apex of the locule (Fig. 42). Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, ovary wall, septa, and placentae, especially in aerenchyma spaces in the latter two regions. Tannin cells are homogeneous and are mostly absent, except for being sparse in the receptacular region. (See Table 2; not illustrated).

Heteranthera reniformis

Placentation is parietal, with three carpels and one locule. The septa are aposeptalous, but barely touch and are coherent at the center; commissures are present at the junction of the septa, typically with a large intercellular space in the center. The placentae consist of two evaginated flanges. Ovules are mostly pleurotropous, numerous, in two rows per flange. The region of ovule attachment extends continuously from the base to the apex of the locule (Fig. 42). Septal

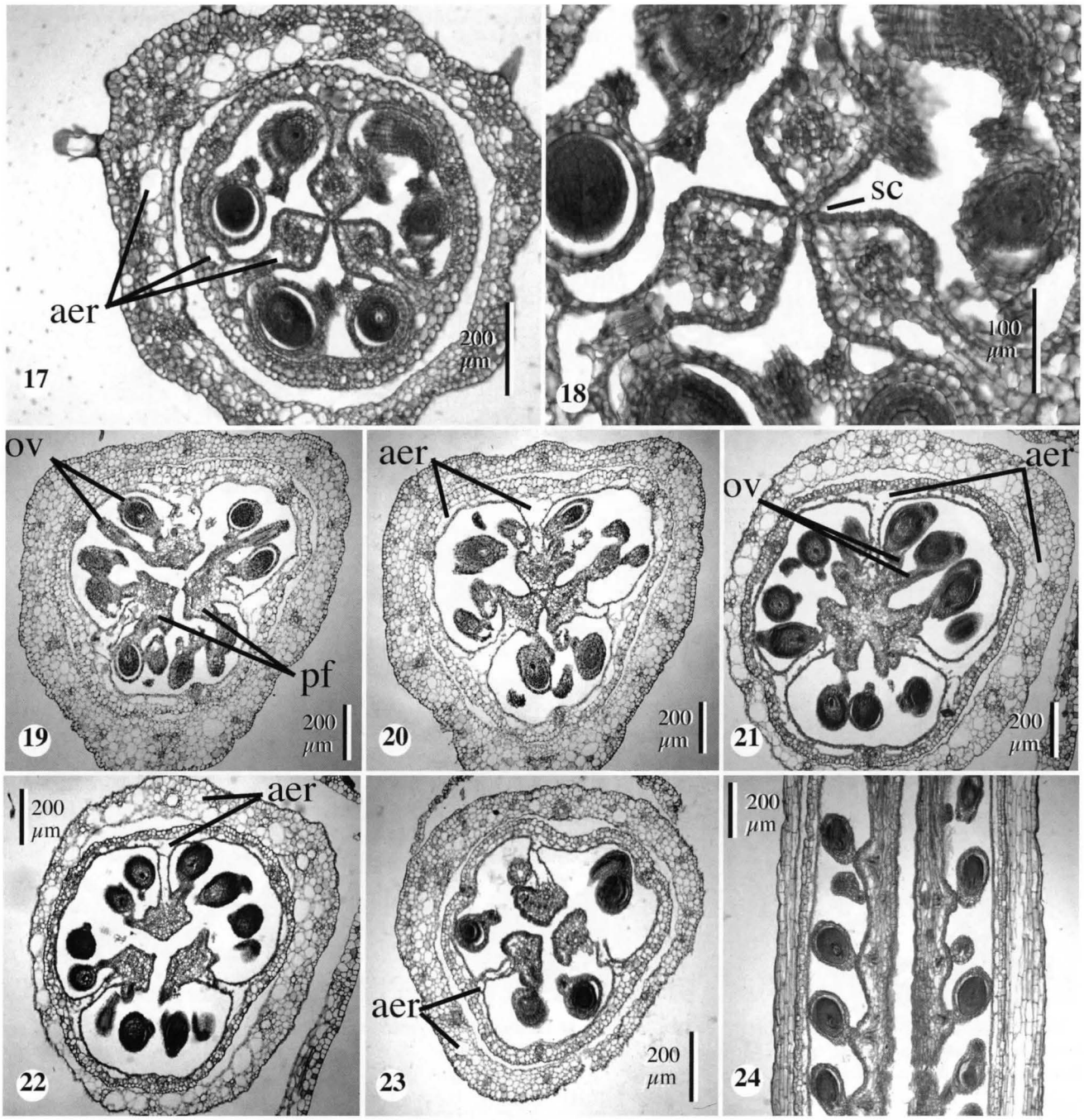

Fig. 17–24.—17–18. *Heteranthera mexicana,* cross section.—17. Middle of ovary, showing aerenchyma (aer).—18. Close-up of barely touching septa, with septal commissures (sc).—19–20. *H. reniformis* cross sections.—19. Base of ovary, showing placental flanges (pf), each with rows of ovules (ov).—20. Apex of ovary, showing aerenchyma (aer) and barely touching septa with septal commissures.—21–22. *H. rotundifolia* cross sections.—21. Base of ovary, showing fused septa; note ovules (ov) and aerenchyma (aer).—22. Apex of ovary, showing unfused septa (hemiseptalous throughout).—23–24. *H. zosterifolia.*—23. Cross section, showing aerenchyma (aer) and unfused septa (aposeptalous).—24. Longitudinal section.

nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, ovary wall, septa, and placentae, especially in aerenchyma spaces in the latter two regions. Tannin cells are homogeneous and are absent except for being very sparse in the receptacular region. (See Fig. 19, 20; Table 2).

Heteranthera rotundifolia

Placentation is axile-parietal with three carpels and three locules at the ovary base, one locule at the apex. The septa are fused from base to middle region of locule (hemiseptalous). Commissures at the junction of the septa are present at the junction of the three fused septa. The placentae consist of two evaginated flanges. Ovules are mostly pleurotropous,

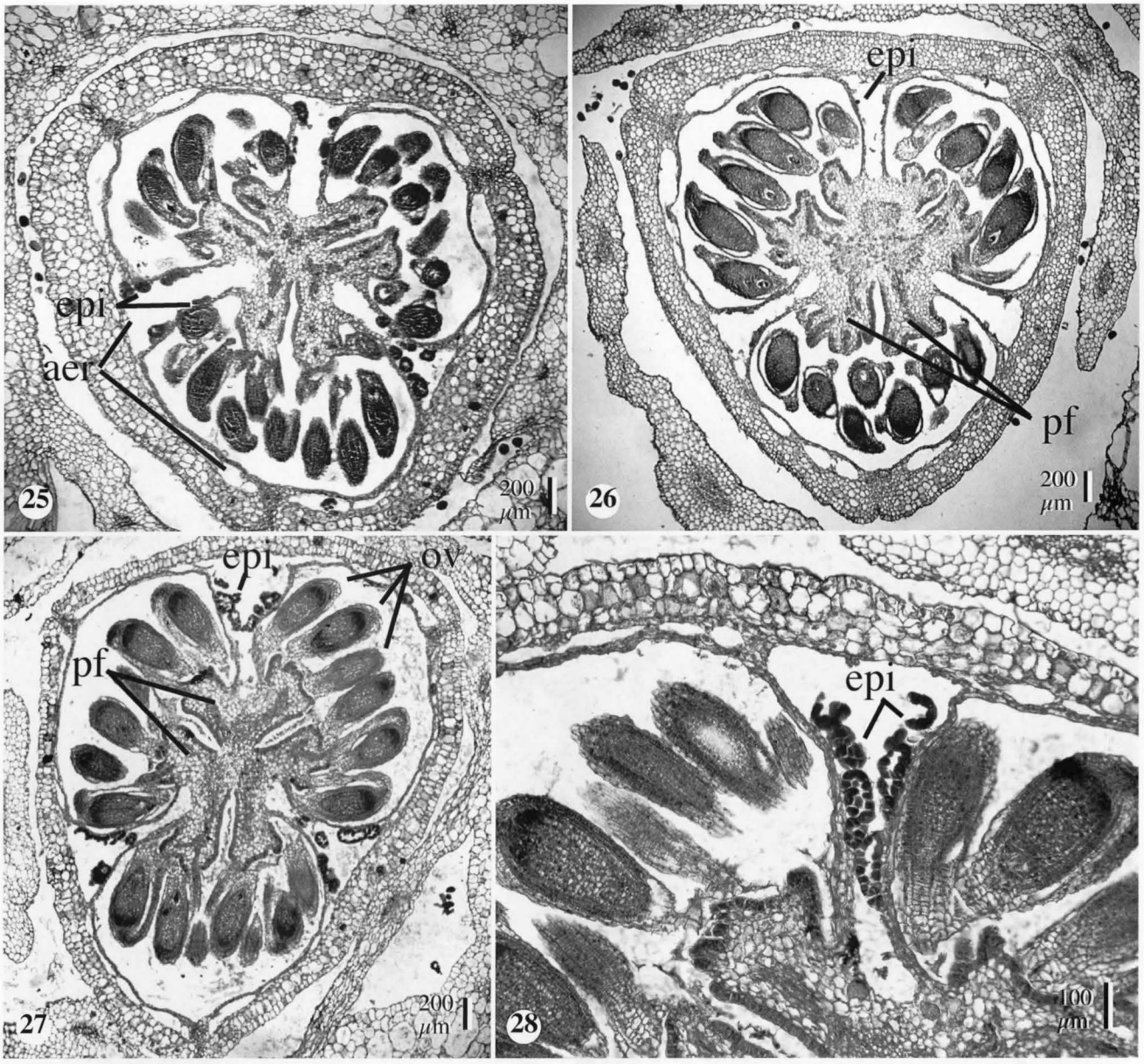

Fig. 25–28.—*Monochoria* species, flower cross sections.—25. *M. hastata.* Note aerenchyma (aer) and epithelial cells (epi).—26. *M. korsikovii,* showing placental flanges (pf) and epithelial cells (epi).—27–28. *M. vaginalis.*—27. Central region of locule, showing synseptalous fusion, placental flanges (pf), and several rows of ovules (ov) per flange. Note epithelial cells (epi).—28. Close-up of epithelial cells (epi).

numerous, in two to three rows per flange. The region of ovule attachment extends continuously from the base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, ovary wall, and septa/placentae junction, especially in aerenchyma spaces. Tannin cells are absent. (See Fig. 21, 22; Table 2).

Heteranthera seubertiana

Placentation is parietal, with three carpels and one locule. The septa are unfused throughout (aposeptalous). Commissures at the junction of the septa are absent (septa not fused). The placentae consist of two slightly evaginated flanges. Ovules are mostly pleurotropous, numerous, in one to two rows per flange. The region of ovule attachment extends continuously from the base to the apex of the locule (Fig. 42). Septal nectaries are absent (Fig. 45). Aerenchyma is present in tissues of the receptacle and septa. Epithelial cells are absent. Calcium oxalate raphides are sparsely distributed in tissues of receptacle, perianth, ovary wall, and septa. Tannin cells are homogeneous and are sparse in the receptacular region, but otherwise absent. (See Fig. 45; Table 2).

Heteranthera zosterifolia

Placentation is parietal, with three carpels and one locule. The septa are unfused throughout (aposeptalous). Commissures at the junction of the septa are absent (septa not fused).

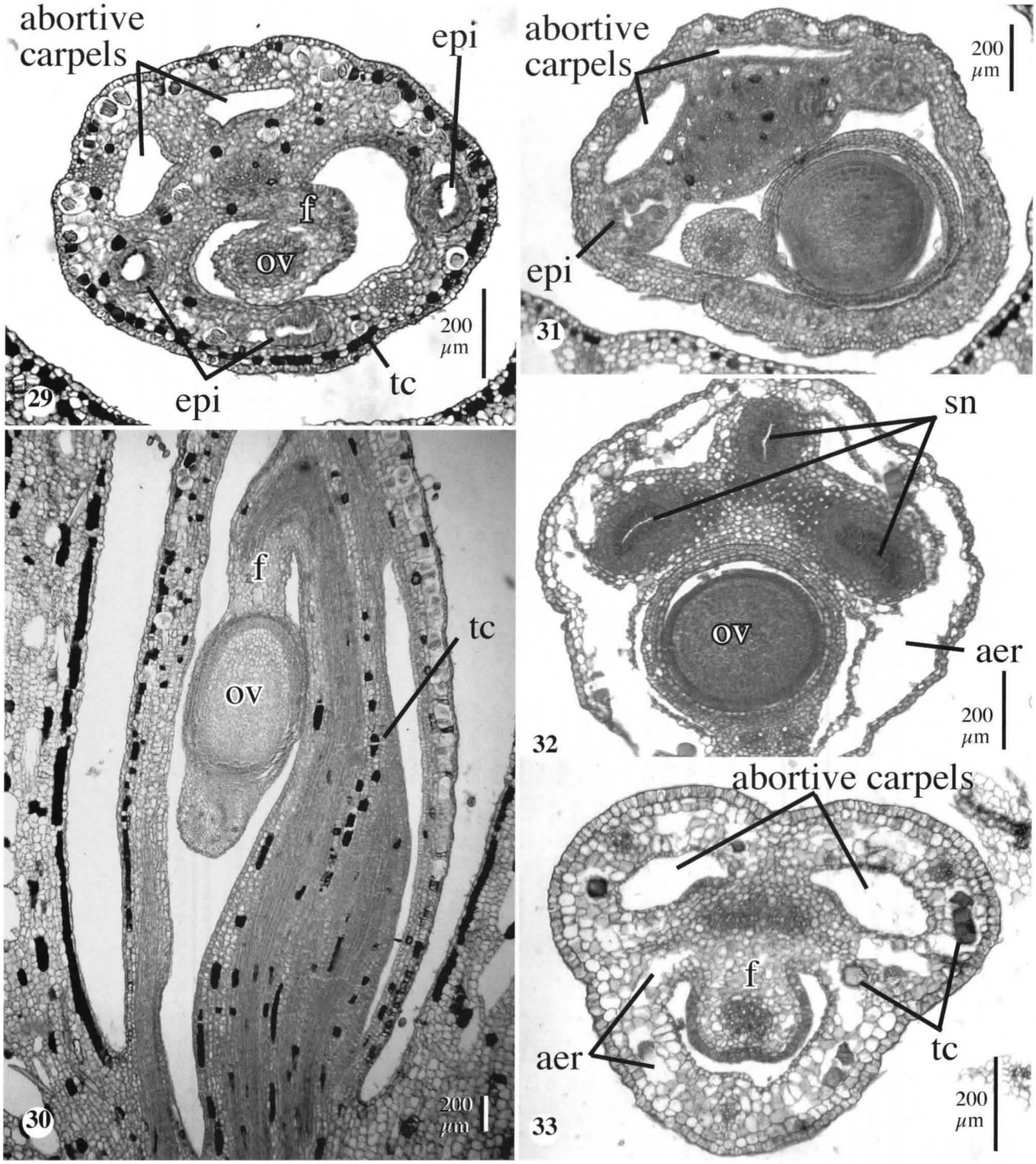

Fig. 29–33.—29–30. *Pontederia cordata* var. *cordata.*—29. Cross section, showing two abortive carpels and epithelial cells (epi). Note also tannin cells (tc), funiculus (f), and apex of ovule (ov).—30. Longitudinal section, showing funiculus (f) and ovule (ov), apically attached; also note numerous tannin cells (tc).—31. *P. sagittata.* Ovary cross section, showing two abortive carpels, functional ovule (ov) and epithelial cells (epi).—32–33. *Reussia subovata,* flower cross sections.—32. Lower ovary region, showing septal nectaries (sn), single functional ovule (ov) with abortive carpels above, and aerenchyma (aer).—33. Upper ovary region, showing funiculus (f), abortive carpels, aerenchyma (aer), and tannin cells (tc).

Table 2. Floral anatomical features in Pontederiaceae and outgroups. For "Ovule row no. per carpel," the number of rows on each side of the placenta are indicated, separated by a "+" sign. Parentheses "( )" indicate a relatively rare condition. For "Placentation (traditional)" and "Septal fusion" an asterisk "*" indicates taxa in which the septa barely touch, being coherent with an evident septal commissure.

Taxon	Carpel number	Locule number	Placentation (traditional)	Placenta position	Placenta evagination	Septal fusion	Ovule no. per ovary	Ovule row no. per carpel
PONTEDERIACEAE:								
Eichhornia crassipes	3	3	axile	continuous	2-flanged	synseptalous	∞	2–4 + 2–4
E. diversifolia	3	3	axile	continuous	2-flanged	synseptalous	∞	2 + 2 (1 + 1)
E. heterosperma	3	3	axile	continuous	2-flanged	synseptalous	∞	1 + 1
E. meyeri	3	3 + 1	axile-parietal	continuous	2-flanged	hemiseptalous	∞	2–3 + 2–3
E. paniculata	3	3 + 1	axile-parietal	continuous	2-flanged	hemiseptalous	∞	2 + 2 (1 + 1)
Heteranthera dubia	2	1	parietal	continuous	slightly 2-flanged	aposeptalous	∞	1 + 1
H. mexicana	3	1	parietal*	continuous	slightly 2-flanged	aposeptalous*	∞	2 + 2
H. multiflora	3	1	parietal	continuous	2-flanged	aposeptalous	∞	2–3 + 2–3
H. reniformis	3	1	parietal*	continuous	2-flanged	aposeptalous*	∞	2 + 2
H. rotundifolia	3	3 + 1	axile-parietal	continuous	2-flanged	hemiseptalous	∞	2–3 + 2–3
H. seubertiana	3	1	parietal	continuous	slightly 2-flanged	aposeptalous	∞	1–2 + 1–2
H. zosterifolia	3	1	parietal	continuous	slightly 2-flanged	aposeptalous	∞	1 + 1
Hydrothrix gardneri	3	1	parietal	continuous	slightly 2-flanged	aposeptalous	∞	1 + 1
Monochoria hastata	3	3	axile	continuous	2-flanged	synseptalous	∞	2–3 + 2–3
M. korsikovii	3	3	axile	continuous	2-flanged	synseptalous	∞	2–3 + 2–3
M. vaginalis	3	3	axile	continuous	2-flanged	synseptalous	∞	2–3 + 2–3
Pontederia cordata var. *c.*	1 + 2	3	apical-axile	apical	unevaginated	synseptalous	1	1—fertile carpel only
P. sagittata	1 + 2	3	apical-axile	apical	unevaginated	synseptalous	1	1—fertile carpel only
Reussia subovata	1 + 2	3	apical-axile	apical	unevaginated	synseptalous	1	1—fertile carpel only
Scholleropsis lutea	3	3 + 1	axile-parietal (?)	continuous	2-flanged	hemiseptalous (?)	∞	?
OUTGROUPS:								
Helmholtzia acorifolia	3	3	axile	continuous	2-flanged	synseptalous	∞	3–4 + 3–4
Philydrum lanuginosum	3	1	parietal	continuous	2-flanged	aposeptalous	∞	4–8 + 4–8
Haemodorum spicatum	3	3	axile	medial	unevaginated	synseptalous	6	1 + 1
Tribonanthes variabilis	3	3	axile	continuous	unevaginated	synseptalous	∞	6–8

Table 2 (continued). Floral anatomical features in Pontederiaceae and outgroups. Symbols: a = androecium, o = ovary, p = perianth, pl = placenta, r = receptacle, s = septa, st = style; P = prismatic crystals, R = raphide crystals, S = styloid crystals; H = homogeneous, G = granular, F = fibrillar; "−" = absent, "+" = present, "?" = unknown or uncertain, n. a. = not applicable. Parentheses "( )" indicate sparse or reduced distribution of a feature. See text for further explanation.

Taxon	Septal commissures	Septal nectaries	Floral aerech. dist. [r, p, o, s]	Epithelial cells	Oxalate crystals	Oxalate crystals [r, p, o, s, pl, a, st]	Tannin cell type	Tannin cell distribution [r, p, o, s, pl, a, st]
PONTEDERIACEAE:								
Eichhornia crassipes	− (?)	+	r, p, o, s	−	R	r, p, o, −, pl, a, ?	H, G	r, p, o, (s), pl, −, st
E. diversifolia	−	−	r, p, −, s	−	R	r, p, o, −, −, −, −	H, F	r, p, o, (s), −, −, st
E. heterosperma	apical	+	r, p, −, s	−	R	−, p, o, −, pl, −, −	F (H)	r, (p, o, s), −, −, st
E. meyeri	−	+	r, p, o, s	−	R	r, p, o, −, −, −, −	G	(r), (p), −, −, −, −, (st)
E. paniculata	apical	+	r, p, o, s	−	R	−, p, o, s, pl, −, −	G	(r), −, −, −, −, −, −
Heteranthera dubia	− (n. a.)	−	r, p, o, s	−	R	r, p, −, −, −, −, −	H, G	r, (p), o, s, pl, a, st
H. mexicana	present throughout	−	r, p, o, s	−	R	r, p, o, s, −, −, −	−	−, −, −, −, −, −, −
H. multiflora	− (n. a.)	−	r, p, o, s	−	R	r, −, o, s, pl, −, −	H	(r), −, −, −, −, −, −
H. reniformis	present throughout	−	r, p, o, s	−	R	r, −, o, s, pl, −, −	H	(r), −, −, −, −, −, −
H. rotundifolia	+	−	r, p, o, s	−	R	r, −, o, s, pl, −,−	−	−, −, −, −, −, −, −
H. seubertiana	− (n. a.)	−	r, −, −, s	−	R	(r, p, o, s), −, −, −	H	(r), −, −, −, −, −, −
H. zosterifolia	− (n. a.)	−	r, p, o, s	−	R	r, p, −, −, −, −, st	H	(r), −, −, −, −, −, −
Hydrothrix gardneri	− (n. a.)	−	r, p, o, s	−	R	r, (p), −, −, −, −, −	H, G	r, −, −, −, −, −, st
Monochoria hastata	−	−	r, p, o, s	+	R	r, p, o, −, −, −, −	H, G	(r), (p), −, −, −, −, −
M. korsikovii	−	−	r, p, o, s	(+)	R	r, p, o, −, −, −, st	H	(r), −, −, −, −, −, −
M. vaginalis	−	−	r, p, o, s	+	R	r, p, o, −, −, −, −	H, G	r, p, (o), s, −, −, st
Pontederia cordata var. *c.*	−	+	r, p, o, −	+	P, R, S	r, p, o, s, −, ?, st	H, G	r, p, o, s, pl, ?, st
P. sagittata	−	+	r, p, o, −	+	P, R, S	r, p, o, s, −, ?, st	H, G, F	r, p, o, s, pl, ?, st
Reussia subovata	−	+	r, p, o, (s)	−	P, R, S	r, p, o, −, −, ?, −	H	r, −, (o), −, −, −, −
Scholleropsis lutea	?	?	?	?	R	−, −, o, s, −, −, −	?	?
OUTGROUPS:								
Helmholtzia acorifolia	−	−	−, −, −, s	−	P, S	r, p, o, s, pl, a, st	H, G, F	r, p, o, s, pl, a, st
Philydrum lanuginosum	−	−	−, −, −, s	−	P, S	r, p, o, s, pl, a, st	H, G, F	r, p, o, s, pl, a, st
Haemodorum spicatum	−	+	−, −, −, −	−	R	r, p, o, s, pl, a, st	−	−, −, −, −, −, −, −
Tribonanthes variabilis	−	+	−, −, −, −	−	R	r, p, o, s, pl, a, st	H, G, F	r, −, o, s, pl, −, −

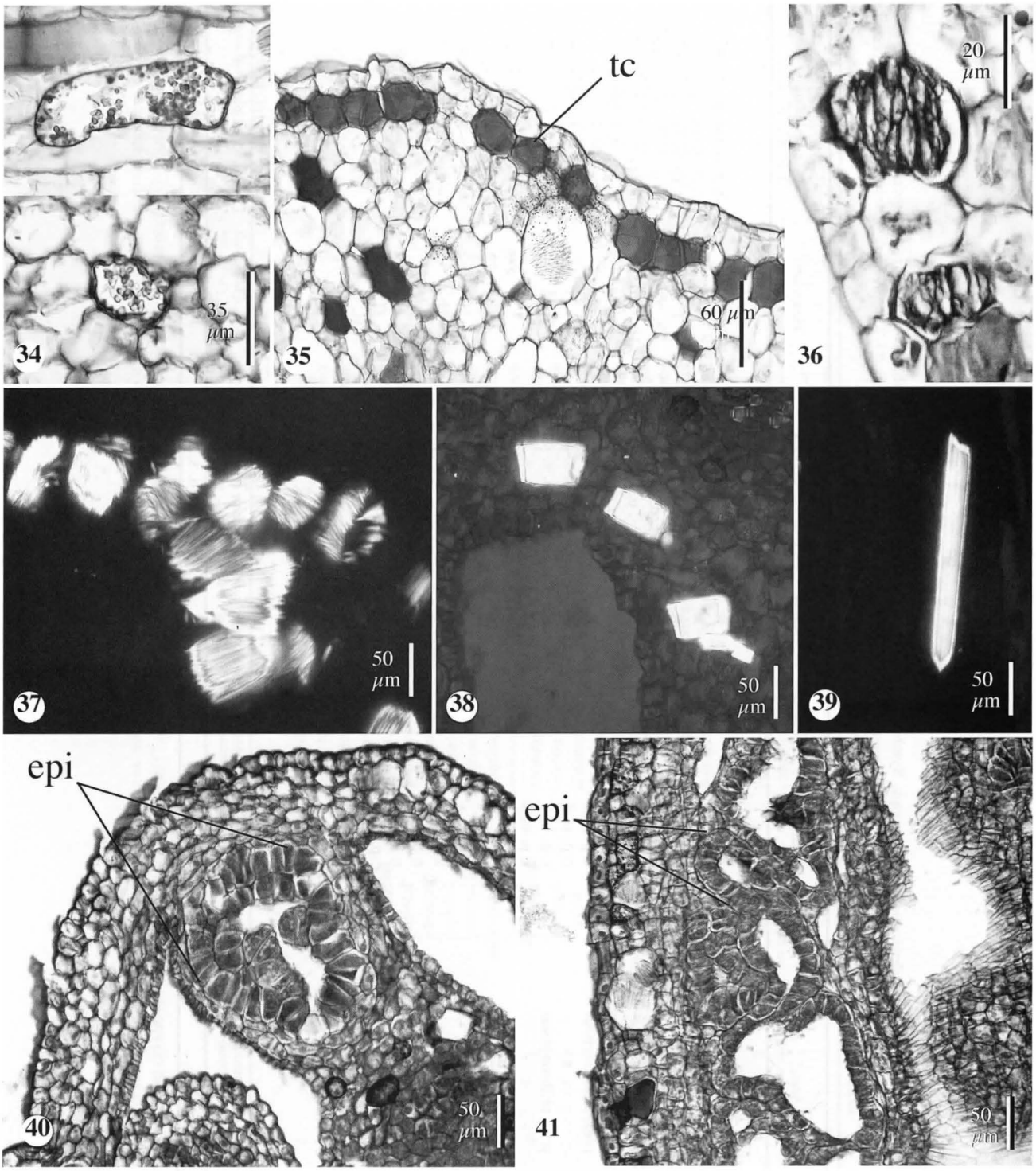

Fig. 34–41.—34–36. Tannin cells.—34. Granular tannin cells, *Pontederia cordata* var. *cordata,* longitudinal section (above) and cross section (below).—35. Homogeneous tannin cells, *P. cordata* var. *cordata.*—36. Fibrillar tannin cells, *Eichhornia heterosperma.*—37–39. Crystals.—37. Raphides, *P. cordata* var. *cordata.*—38. Prismatics, *P. sagittata.*—39. Styloids, *P. sagittata.*—40–41. Epithelial cells (epi).—40. *P. sagittata,* ovary wall cross section.—41. *P. cordata* var. *cordata,* ovary wall longitudinal section.

The placentae consist of two slightly evaginated flanges. Ovules are mostly pleurotropous, numerous, in one row per flange. The region of ovule attachment extends continuously from the base to the apex of the locule (Fig. 42). Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, perianth, and style. Homogeneous tannin cells are very sparsely present in the receptacular region, otherwise absent. (See Fig. 23, 24; Table 2).

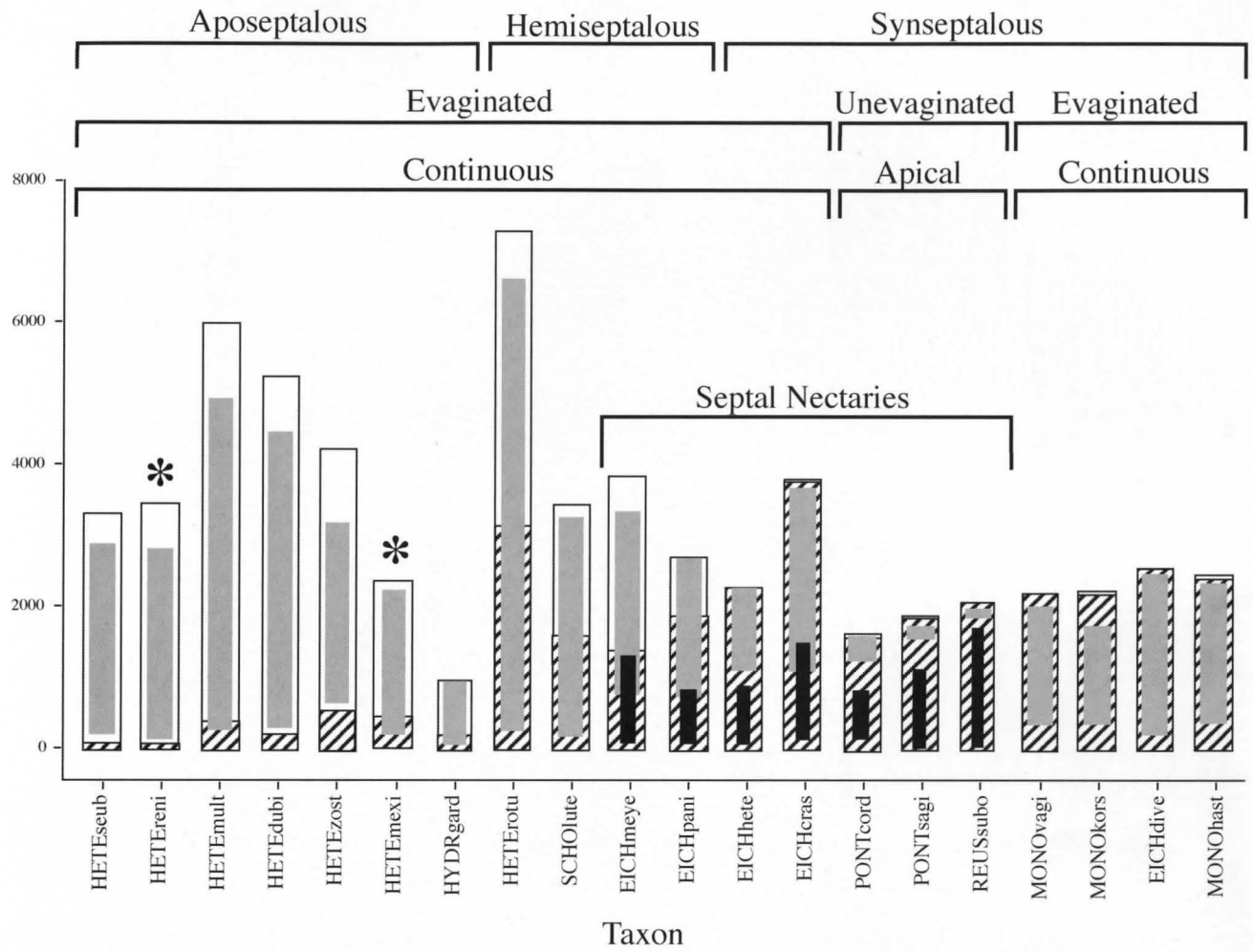

Fig. 42.—Diagram showing vertical spatial relationships of septa (fused regions shown by hatch marks, unfused by white), placental tissue (gray), and septal nectaries (black). Categories correspond to septal fusion (aposeptalous, hemiseptalous, and synseptalous), placental type (evaginated and unevaginated), placental position (continuous and apical), and septal nectary presence. An asterisk (*) symbol indicates two taxa that have apically coherent septae, with commissures. Taxa abbreviations correspond to genera (all capital letters) and specific epithets (small case letters); see Materials and Methods.

Hydrothrix gardneri

Flowers are paired, each with one stamen, the pair surrounded by bracts. Placentation is parietal, with three carpels and one locule. The septa are unfused throughout (aposeptalous). Commissures at the junction of the septa are absent (septa not fused). The placentae consist of two slightly evaginated flanges. Ovules are mostly pleurotropous, numerous, in one row per flange. The region of ovule attachment extends continuously from the base to the apex of the locule (Fig. 42). Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are absent. Calcium oxalate raphides are distributed in tissues of the receptacle, sparse in perianth and surrounding bracts, absent elsewhere. Tannin cells are homogeneous and granular and are distributed in tissues of the receptacle and style and in the surrounding bracts. (See Fig. 16; Table 2).

Monochoria hastata

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous), narrowly fused near the apex. Commissures at the junction of the septa are not present. The placentae consist of two evaginated flanges. Ovules are mostly pleurotropous, numerous, in two to three rows per flange. The region of ovule attachment extends continuously from the base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are present within the septal aerenchyma. Calcium oxalate raphides are distributed in tissues of the receptacle, perianth, and ovary wall. Tannin cells are homogeneous and granular and are distributed sparsely in tissues of the receptacle and perianth. (See Fig. 25; Table 2).

Monochoria korsikovii

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are not present. The placentae consist of two evaginated flanges. Ovules are mostly pleurotropous, numerous, in two to three rows per flange. The region of ovule attachment extends continuously from the

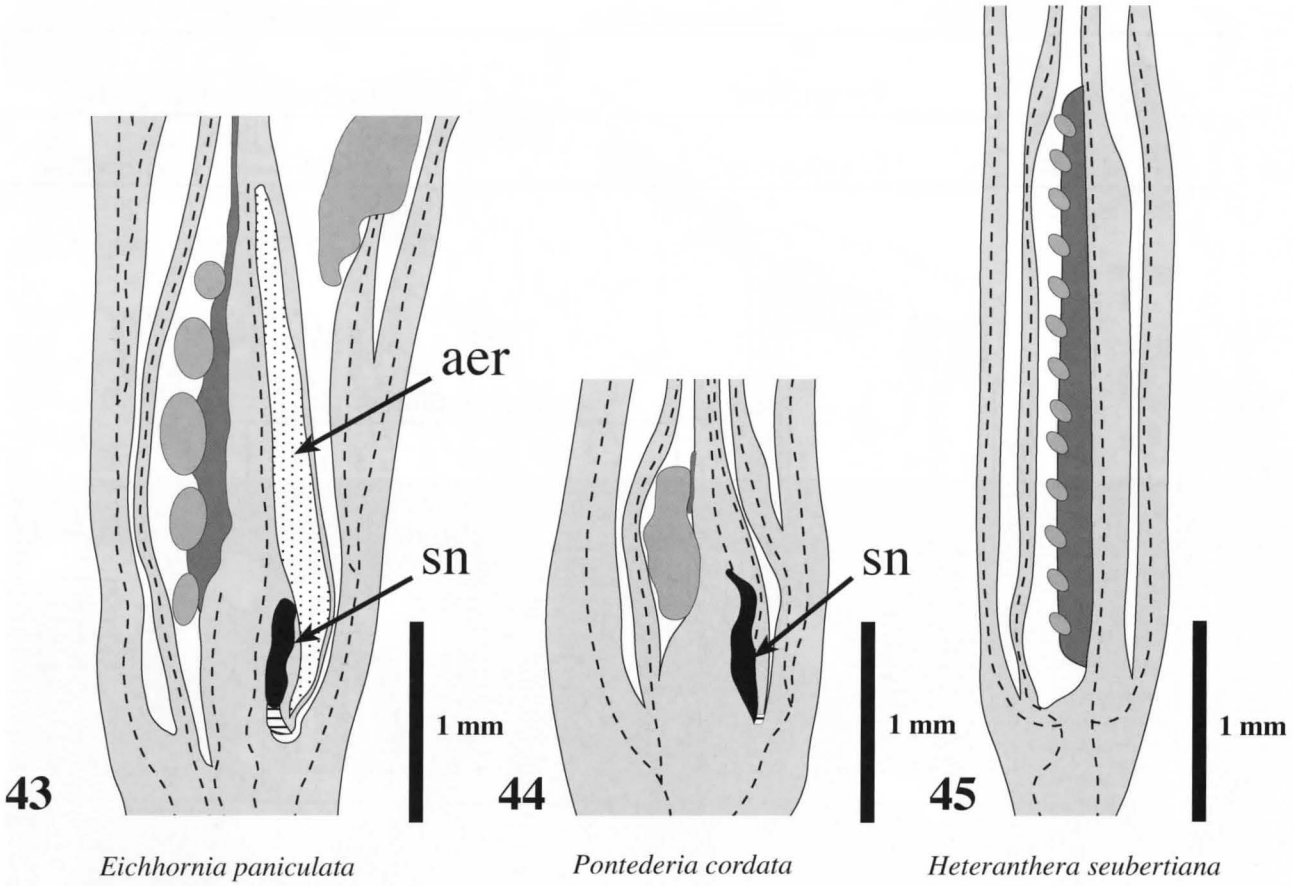

Fig. 43–45.—Diagrams of flowers in longitudinal-section.—43. *Eichhornia paniculata.*—44. *Pontederia cordata* var. *cordata.*—45. *Heteranthera seubertiana.*

base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are present but reduced in septal aerenchyma; structures resembling epithelial cells occur at the base of ovules. Calcium oxalate raphides are distributed in tissues of the receptacle, perianth, ovary wall, and style. Tannin cells are homogeneous and are distributed sparsely in tissues of the receptacle. (See Fig. 26; Table 2).

Monochoria vaginalis

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are not present. The placentae consist of two evaginated flanges. Ovules are mostly pleurotropous, numerous, in two to three rows per flange. The region of ovule attachment extends continuously from the base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and septa. Epithelial cells are present within septal aerenchyma. Calcium oxalate raphides are distributed in tissues of the perianth, receptacle, and ovary wall. Tannin cells are homogeneous and granular and are distributed in tissues of the receptacle, perianth, ovary wall (sparsely), septa (central column region), and style. (See Fig. 27, 28; Table 2).

Pontederia cordata *var.* cordata

Placentation is apical-axile, with one fertile (median-anterior) carpel, two sterile (latero-posterior) carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are not present. The placentae are unexpanded and effectively absent. The single ovule is epitropous, with the funiculus curved upward 180°. The region of ovule attachment is at the locule apex only. Septal nectaries are present in the lower portion of the locule (Fig. 42, 44). Aerenchyma is present in tissues of the receptacle, perianth, and ovary wall. Epithelial cells are present in aerenchyma of ovary walls and at the junction of ovary wall and septa. Calcium oxalate raphide, prismatic, and styloid crystals are distributed in tissues of the receptacle, perianth, ovary wall, septa, and style; prismatic and styloid crystals are rare in tissues of the gynoecium. Tannin cells are homogeneous and granular and are distributed in tissues of the receptacle, perianth, ovary wall, septa, placentae, and style. (See Fig. 29, 30, 44; Table 2).

Pontederia sagittata

Placentation is apical-axile, with one fertile (median-anterior) carpel, two sterile (latero-posterior) carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are not present. The placentae are unexpanded and effectively absent. The single

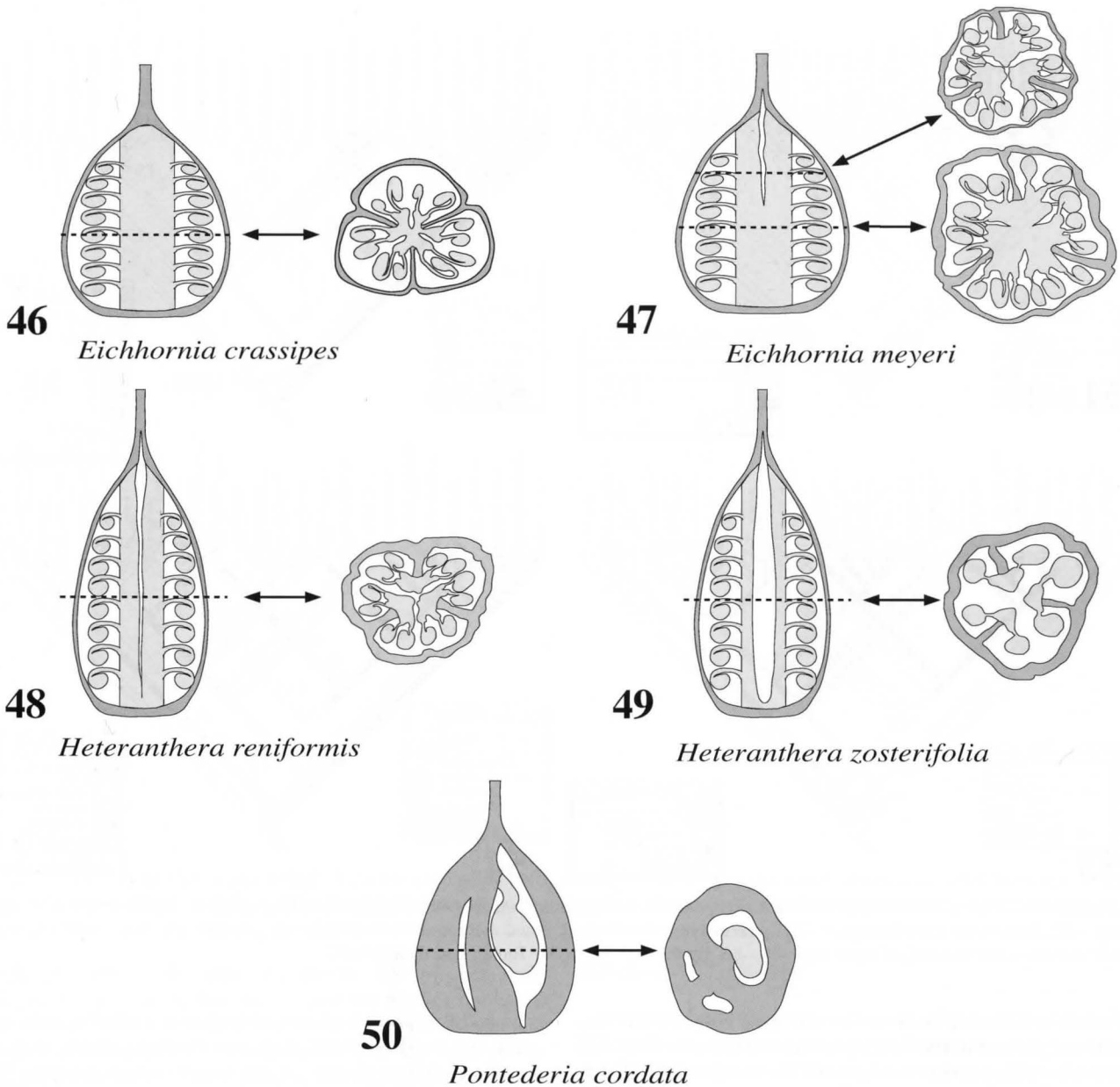

Fig. 46–50.—Diagrams representing modified placentation types.—46. Synseptalous with medial ovule position ("axile").—47. Hemiseptalous with medial ovule position ("axile" below, "parietal" above).—48–49. Aposeptalous with medial ovule position ("parietal").—50. Synseptalous with apical ovule position ("axile").

ovule is epitropous, with the funiculus curved upward 180°. The region of ovule attachment is at the locule apex only. Septal nectaries are present in the lower portion of the locule (Fig. 42). Aerenchyma is present in tissues of the receptacle, perianth, and ovary wall. Epithelial cells are present within the aerenchyma of ovary walls and at the junction of ovary wall and septa. Calcium oxalate raphide, prismatic, and styloid crystals are distributed in tissues of the receptacle, perianth, ovary wall, septa, and style. Prismatics and styloids are common in inner cell layers of the perianth tube and the column/septa regions and are absent in stylar region. Raphides are more common in outer perianth tube tissues and in the ovary wall. Tannin cells are homogeneous, granular, and fibrillar and are distributed in tissues of receptacle, perianth, ovary wall, septa, placentae, and style. In the perianth tube, the homogeneous type makes up a hypodermis in the outer layers; granular and fibrillar types comprise a hypodermis of the inner layers. Granular tannin cells are more common in tissues of the gynoecium. (See Fig. 31; Table 2).

Reussia subovata

Placentation is apical-axile, with one fertile (median-anterior) carpel, two sterile (latero-posterior) carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are not present. The placentae are unexpanded and effectively absent. The single ovule is epitropous; the funiculus is curved 180°. The region

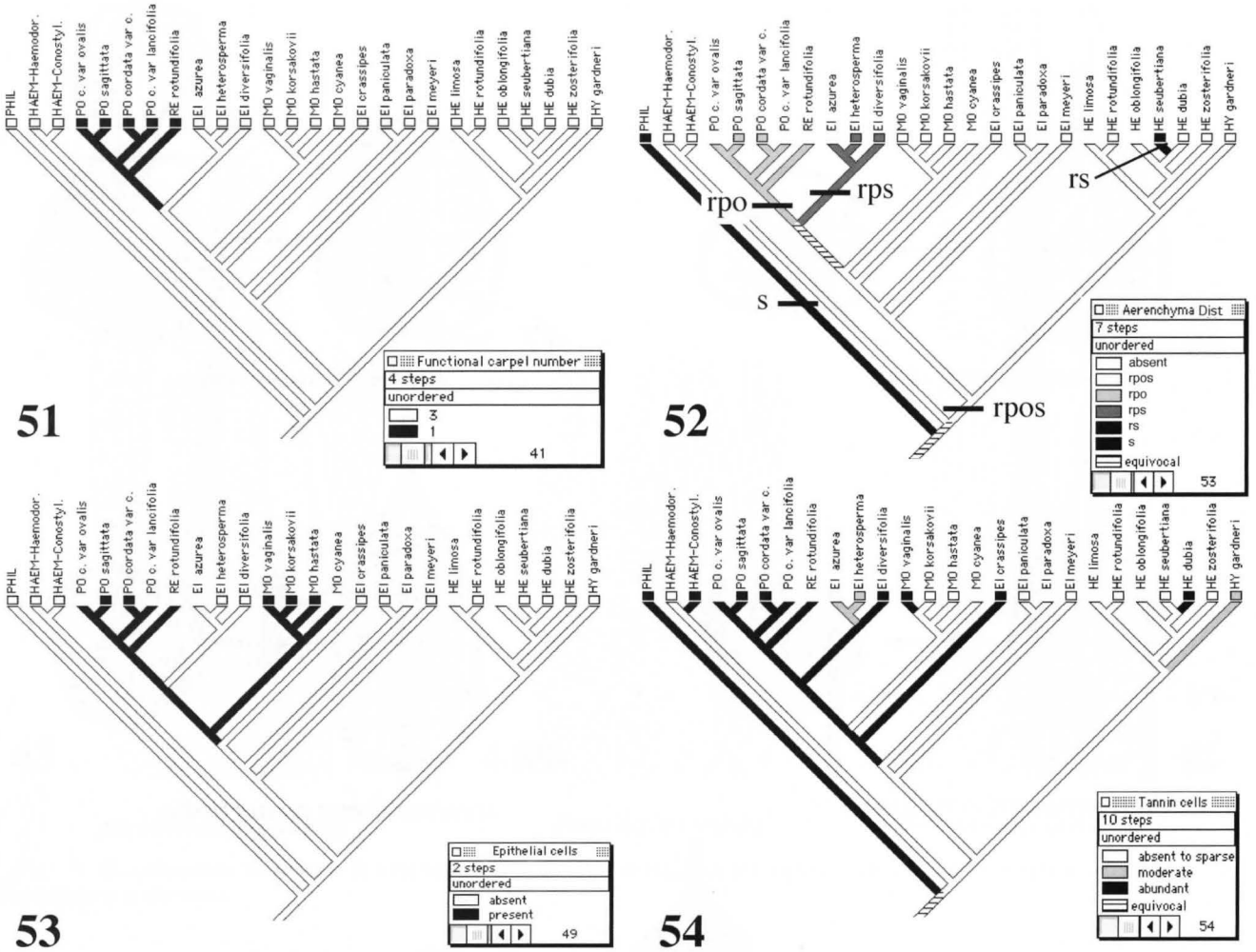

Fig. 51–54.—Plot of floral anatomical characters onto a combined chloroplast DNA cladogram of Graham et al. 1998 (their Fig. 2, right side).—51. Functional carpel number.—52. Aerenchyma distribution, noted as present in receptacle (r), perianth (p), ovary wall (o), septa (s) or various combinations of these regions.—53. Epithelial cells.—54. Tannin cell distribution.

of ovule attachment is at the locule apex only. Septal nectaries are present in the lower portion of the locule (Fig. 42). Aerenchyma is present in tissues of the receptacle, perianth, ovary wall, and junction of septa with ovary wall. Epithelial cells are absent. Calcium oxalate raphide, prismatic, and styloid crystals are distributed in tissues of the receptacle, perianth, and ovary wall. Raphides are especially common in aerenchyma spaces; prismatic and styloid crystals are rare in the gynoecium. Tannin cells are homogeneous and are distributed in tissues of the receptacle, sparsely in the ovary wall. (See Fig. 32, 33; Table 2).

Schlleropsis lutea

Placentation is axile-parietal, with three carpels and three locules at the ovary base, one at the apex, the septa fused from the base to middle region of locule (hemiseptalous). The presence or absence of commissures at the junction of the septa is unknown. The placentae consist of two evaginated flanges. Ovules are numerous, the region of ovule attachment extending continuously from the base to the apex of the locule. Septal nectaries were not observed, therefore their presence or absence could not be confirmed. The presence and distribution of aerenchyma and epithelial cells also could not be determined. Calcium oxalate raphide crystals are distributed in tissues of the ovary wall and septa. The presence/absence of tannin cells could not be determined. In general, the poor material available for this species warrants a future reexamination. (Not illustrated; see Table 2).

Haemodorum spicatum

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are absent. The placentae are swollen but unevaginated, lacking flanges. Ovules are hypotropous, two per carpel, in two rows. The region of ovule attachment is medial. Septal nectaries are present. Aerenchyma and epithelial cells are absent. Only raphide crystals are present, distributed in all tissues of the flower. Tannin cells are absent. (Not illustrated; see Table 2).

Tribonanthes variabilis

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous). Commissures

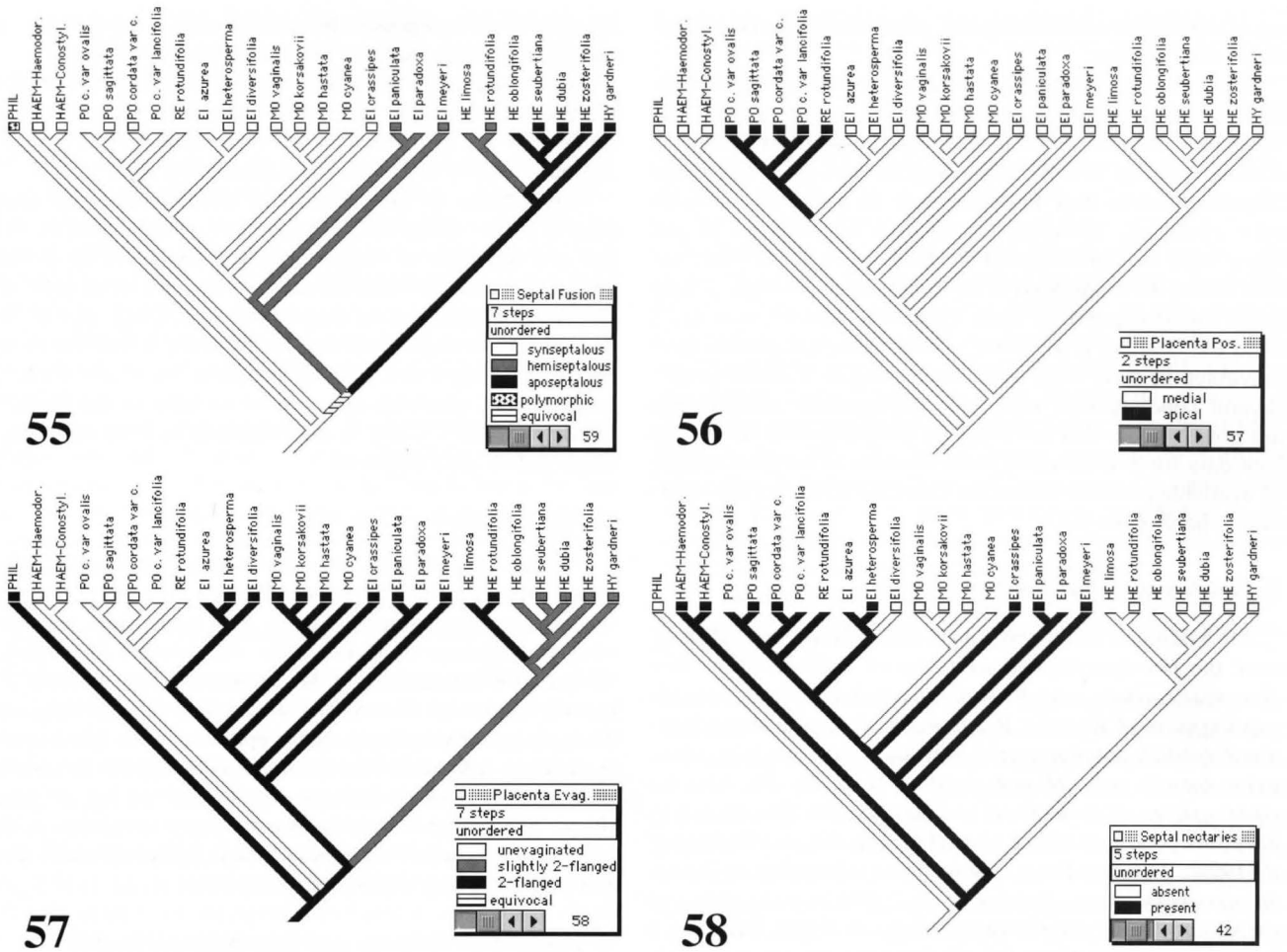

Fig. 55–58.—Plot of floral anatomical characters onto a combined chloroplast DNA cladogram of Graham et al. 1998 (their Fig. 2, right side).—55. Placentation, septal fusion.—56. Placentation, ovule position.—57. Placentation, placenta evagination.—58. Septal nectaries.

at the junction of the septa are absent. The placentae are unevaginated, lacking flanges. Ovules are pleurotropous, in six to eight rows per carpel. The region of ovule attachment extends continuously from near the base to the apex of the locule. Septal nectaries are present. Aerenchyma and epithelial cells are absent. Only raphide crystals are present, distributed in all tissues of the flower. Tannin cells are homogeneous, granular, and fibrillar, and are distributed in the receptacle, ovary wall, septa, and placental tissues. (Not illustrated; see Table 2).

Helmholtzia acorifolia

Placentation is axile, with three carpels and three locules. The septa are fused throughout (synseptalous). Commissures at the junction of the septa are absent. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in three to four rows per flange. The region of ovule attachment extends continuously from near the base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present only in tissues of the septa. Epithelial cells are absent. Prismatic and styloid crystals only are distributed in all tissues of the flower. Tannin cells are homogeneous, granular, and fibrillar, and are distributed in all tissues of the flower. (Not illustrated; see Table 2).

Philydrum lanuginosum

Placentation is parietal, with three carpels and one locule. The septa are unfused throughout (aposeptalous). Commissures at the junction of the septa are absent. The placentae consist of two evaginated flanges per carpel. Ovules are pleurotropous, numerous per carpel, in four to eight rows per flange. The region of ovule attachment extends continuously from near the base to the apex of the locule. Septal nectaries are absent. Aerenchyma is present only in tissues of the septa. Epithelial cells are absent. Prismatic and styloid crystals only are distributed in all tissues of the flower. Tannin cells are homogeneous, granular, and fibrillar, and are distributed in all tissues of the flower. (Not illustrated; see Table 2).

DISCUSSION

The above observations of the floral anatomy of Pontederiaceae demonstrate considerable variation within the family that may be valuable in systematic analyses. The observations of Burton and Simpson (1996) and of Strange et al. (2004) were confirmed from the present study, but with additional general characters and a higher taxon sampling. In addition, this study has led us to consider redefinition of

some commonly used characters, particularly that of placentation (see below).

The following is a summary of the significant floral anatomical characters. As mentioned earlier, we use a most parsimonious tree derived from combined chloroplast DNA data of Graham et al. (1998), along with the two cited outgroups (Haemodoraceae and Philydraceae), to evaluate character state evolution. However, some taxa investigated in our study were not investigated by Graham et al. (1998), and vice versa; the cladogram of Pontederiaceae portrays all taxa (with the exception of their unknown "*Eichhornia* sp.") from the Graham et al. (1998) study. Although doubts were expressed in their study as to the rooting of Pontederiaceae, several concordances with their phylogenetic relationships are noted. (Note that for many of the characters below, we lack data for *Scholleropsis lutea* because of the poor quality of available material; character states are noted for this taxon only where observed.)

1. Functional Carpel Number (Fig. 51)

All examined family members have three carpels. However, the two investigated members of *Pontederia, P. cordata* var. *cordata,* and *P. sagittata,* and the single investigated species of *Reussia, R. subovata,* all have a single functional median-anterior carpel with the other two latero-posterior carpels reduced and abortive (Fig. 29, 31, 33). All other species of *Pontederia* and *Reussia* are also known to have this condition (these plotted on Fig. 51; see Graham et al. 1998). This condition is sometimes referred to as "pseudomonomery" (e.g., Strange et al. 2004) because only one carpel and locule are obvious at floral maturity; however, it is evident from sections that all three are present, with only one being functional (i.e., containing an ovule).

Of the outgroups, only *Barberetta* Harv. of Haemodoraceae has a single functional carpel, but this is posterior in position (Simpson 1990). This difference in fertile carpel position plus the relatively terminal phylogenetic position of *Barberetta* support the notion that the single fertile carpel in members of the two families was independently derived.

2. Floral Aerenchyma Distribution (Fig. 52)

Aerenchyma tissue is found in all observed members of Pontederiaceae, the taxa differing only in the location within the flower. Within the family, all taxa examined have aerenchyma in the receptacle region (Table 2); the majority of taxa examined have aerenchyma in major floral parts: perianth (e.g., Fig. 17), ovary wall (e.g., Fig. 6), and septal/placental regions (e.g., Fig. 9; see Table 2). However, two species of *Eichhornia* (*E. diversifolia* and *E. heterosperma*) lack aerenchyma in the ovary wall, having it only in the receptacle, perianth, and septa (rps). The two *Pontederia* species studied possess aerenchyma in the receptacle and perianth, and to a lesser extent in the ovary wall (rpo), but with no aerenchyma occurring in the septa and adjacent placental region. *Reussia subovata,* not studied by Graham et al. 1998, and not plotted in Fig. 52, is similar to these in having aerenchyma in the receptacle, perianth, and ovary wall and only very sparsely present in the septa (Table 2). *Heteranthera seubertiana* is distinctive in having aerenchyma only in the receptacle and septa/placental regions (rs) and lacking it in both the ovary wall and perianth (Fig. 52).

In the outgroups, floral aerenchyma is found only in Philydraceae (two of four species investigated), restricted to the septa only and not found in the receptacular tissue. Aerenchyma is absent in all observed Haemodoraceae.

Aerenchyma is an anatomical characteristic of many aquatic plants (Esau 1965), presumably functioning in storage and diffusion of oxygen reserves and possibly in floatation. Because Pontederiaceae flowers are emergent, the presence of aerenchyma may not be functional, per se, but could be a developmental transfer from the formation of aerenchyma in vegetative parts of the plant. However, it is also possible that aerenchyma could be present in the fruits of family members, where it may function in fruit flotation, a likely aid in seed dispersal.

3. Floral Epithelial Cells (Fig. 53)

Curious aggregations of cells termed "epithelial cells" (after Strange et al. 2004; see Fig. 40, 41) were observed inside aerenchymatous tissue in five taxa: three (of three observed) species of *Monochoria—M. hastata, M. korsakovii* (scantily present), and *M. vaginalis*—and the two observed species of *Pontederia, P. cordata* var. *cordata,* and *P. sagittata.* Interestingly, these epithelial cells were absent in *Reussia subovata.* The epithelial cells appear as laminar proliferations, which become convoluted during development. The adaptive significance of these structures is unknown and requires additional study. Epithelial cells were not present in any studied outgroup taxa.

4. Tannin Cell Presence and Distribution (Fig. 54)

Distinctive floral tannin cells were observed (at least scantily) in almost all taxa of Pontederiaceae. The types of tannin cells vary, being either granular, in which the cells contain granular tannin contents adjacent to the inside of the cell wall (Fig. 34), homogeneous, in which the vacuoles are filled with translucent, safranin-staining tannins (Fig. 35), or somewhat fibrillar in appearance (Fig. 36). However, these types, when more than one are present, often appear to intergrade. Thus, tannin cell type was not reconstructed on the cladogram.

The distribution of tannin cells among various floral tissues is variable in the family and somewhat difficult to quantify (Table 2). Only in two species, *Heteranthera mexicana* and *H. rotundifolia,* were tannin cells not observed at all. However, tannin cells were essentially absent in eight other taxa, being very sparsely present only in the receptacular, perianth, and stylar region of *Eichhornia meyeri* and very scantily present in the receptacular region only of *Eichhornia paniculata, Heteranthera multiflora, H. reniformis, H. seubertiana, H. zosterifolia, Monochoria hastata* (also scantily present in the perianth), and *M. korsikovii.* The distribution of tannin cells in the other investigated species varies somewhat. Tannin cells are abundantly found in the majority (four to six) of floral tissue regions in *Eichhornia crassipes, E. diversifolia, Heteranthera dubia, Monochoria vaginalis, Pontederia cordata* var. *cordata,* and *P. sagittata.* Tannin cells are moderately distributed (found in any significant quantities in only one or two floral tissue regions) in *Eich-*

hornia heterosperma, Hydrothrix gardneri, and *Reussia subovata.* (*Schlleropsis lutea* could not be observed for this feature because of the poor quality of material available.)

Tannin cells were observed in the floral tissues of some Haemodoraceae and in all Philydraceae. The presence or absence of tannin cells is phylogenetically significant in Haemodoraceae, as all members of the subfamily Conostylidoideae possess them and all examined members of subfamily Haemodoroideae lack them (Simpson 1990; erroneously termed "sclereids"). The phylogenetic significance of floral tannin cells in monocots as a whole has not yet been investigated. Although very few taxa have been examined to date, the utility of this feature shows promise as a systematic character.

The adaptive significance of floral tannin cells is not known. As with vegetative tannin cells, they may function to deter herbivory. It is also suspected that tannin cells may function to add hardness to floral tissues, e.g., in the anther connective regions of members of Haemodoraceae and Philydraceae.

5. Crystal Type and Distribution (Fig. 37–39)

All examined members of Pontederiaceae have raphide crystals (Fig. 37) that are birefringent in polarized light and presumably composed of an oxalate salt. Prismatic (Fig. 38) or styloid crystals (Fig. 39), which differ from raphides in being composed of a single crystal (either rectanguloid or spindle-shaped, respectively), are found within the family only in the two investigated members of *Pontederia* and the single investigated species of *Reussia.* Prismatic and styloid crystals appear to intergrade and are treated as a single "type" here. (This character was not plotted, but for Pontederiaceae is presumed to be similar to Fig. 51.)

Raphide crystals were observed in all outgroups. Prismatic/styloid crystals, however, were observed only in all investigated members of Philydraceae.

6. Placentation (Fig. 55–57)

Placentation has long been used as a descriptive character in plant systematics. However, traditional placentation types encompass a number of discrete features and are better subdivided into separate characters that more accurately assess homology. Part of the difficulty with the term "placentation" is in being correlated with the presence, absence, or fusion of septa. In addition, the term can refer to either of two things: (1) the point of attachment of one or more ovules, in which case it refers to a region, without allusion to the morphology of tissue; or (2) the morphology of the placental tissue to which one or more ovules are attached.

We propose a recoding of the character "placentation" to take into account three separate developmental processes: (1) the fusion or lack thereof of septa during carpel development; (2) the positioning of placental tissue relative to the locule(s) of the ovary; and (3) the relative outgrowth (evagination) of placental tissue.

6A. Septal fusion (Fig. 55).—This refers to whether or not the septa (if present) are centrally fused and, if so, the vertical extent of that fusion. Three character state terms are proposed to categorize septal fusion, although additional terms may be needed in other groups: (i) synseptalous (= septa "connate" or "fused"), if the septa are fused the full length of the ovary locules (Fig. 46, 50); (ii) aposeptalous (= septa "distinct" or "unfused"), if the septa are not fused at all or only very little, generally at the extreme base of the ovary (Fig. 48, 49); and (iii) hemiseptalous (= septa "half-connate" or "half-fused"), if the septa are fused only near the base and not fused at the apex of the ovary (Fig. 47).

In Pontederiaceae, nine of the investigated species are synseptalous (*Eichhornia crassipes* being almost hemiseptalous), seven are aposeptalous (although in two species, *Heteranthera mexicana* and *H. reniformis,* the septa often barely touch but have a commissure between them), and four are clearly hemiseptalous (except *Eichhornia meyeri,* in which the septa are fused only at the lower ¼ of the locules; Table 2; Fig. 55, 42). Septal fusion corresponds with the traditional placentation types of axile, parietal, and axile-parietal. However, it is more precise as to *what* is being described and allows for the description of additional aspects of features typically included in placentation.

All members of Haemodoraceae are synseptalous. Philydraceae are polymorphic with respect to this character. (See Fig.55; Table 2).

6B. Placenta position (Fig. 56).—This feature refers to the placement of placentae or ovules relative to the ovary and/or septa, regardless of the morphology of the placental tissue (below). We propose, for the group studied, two character states that take into account a number of ovule attachment positions: (i) continuous, if the ovules are attached in the middle region of the locule(s), typically extending almost the entire length of the locule; and (ii) apical, if ovules are attached at the apex of the locule(s). Almost all taxa examined have a continuous placenta position. Only the species of *Pontederia* and *Reussia* have an apical placental position (Graham et al. 1998; see also Table 2; Fig. 55). The species of *Eichhornia* examined have a somewhat subapical placenta position, the tissue being in the upper ¾ to ½ of the locule; however, this is probably correlated with the presence of basal septal nectaries; see Fig. 42, below. All of the outgroups have a continuous (to medial) ovule attachment.

6C. Placenta evagination (Fig. 57).—This feature describes the morphology of any differentiated outgrowth of tissue, arising from the ovary wall or septa, to which one or more ovules are attached. This character describes the outgrowth of, for example, ridges or expanded pads of tissues. Placenta morphology would likely have states customized for a particular group of study.

For Pontederiaceae, we propose the following character states for this character: (i) unevaginated, if the placental tissue does not form expanded ridges of tissue (e.g., Fig. 29); (ii) 2-flanged, if the placental tissue is evaginated to form two prominent vertical ridges of tissue per carpel that extend from the ends of septa (if aposeptalous) or from the central column of tissue (if synseptalous; see Fig. 8); (iii) slightly 2-flanged, if these evaginations are scarcely present (e.g., Fig. 23).

From our observations, all members of Pontederiaceae have evaginated placentae to some degree except for the investigated species of *Pontederia* and *Reussia.* The lack of expanded placentae in these two genera is certainly corre-

lated with their reduced number of ovules (see below). All investigated Philydraceae have a 2-flanged placental evagination; however, Haemodoraceae lack placental evaginations.

In summary, the inadequacy of traditionally termed placentation types is evident. For example, using traditional placentation terms, both *Eichhornia crassipes* (Fig. 46) and *Pontederia cordata* var. *cordata* (Fig. 50) are typically described as "axile" (e.g., as in Cook 1998), and *Eichhornia meyeri* (Fig. 47) might be termed "axile-parietal." However, *Eichhornia crassipes* (Fig. 46) and *Pontederia cordata* var. *cordata* (Fig. 50) are similar in only one of our revised characters, each having fused septa (termed synseptalous in our system), and differ in two characters: ovule attachment position (medial vs. apical) and placental morphology (evaginated vs. unevaginated). On the other hand, these two *Eichhornia* species are identical in these latter two features and differ only in septal fusion (aposeptalous in *E. meyeri*). Note also that, even though these two *Eichhornia* species differ in septal fusion, their overall septal morphology is rather similar. In fact, the septa of *E. meyeri* almost touch at the center of the ovary (Fig. 11, 12, 47). This similarity in the two *Eichhornia* species may allude to their being functionally similar with regard to fruit dehiscence and seed dispersal. *Pontederia* spp. and *Eichhornia* spp. also differ in ovule number and fertile carpel number, features that may be correlated with our subdivision of placentation as septal fusion, ovule attachment position, and placenta morphology; see below.

7. Ovule Number (Table 2)

All examined family members have numerous (more than ten) ovules per carpel, except for the same two investigated members of *Pontederia* and the single investigated species of *Reussia,* which have a single ovule in one functional carpel. This reduced ovule number is obviously correlated with functional carpel number and with fruit type; *Pontederia* and *Reussia* have single-seeded nutlets/achenes in an anthocarp, whereas all other Pontederiaceae have capsules. Of Pontederiaceae with multiple ovules per carpel (and therefore a 2-flanged or slightly 2-flanged placenta) all have at least two rows of ovules, one on each of the flanges (e.g., "1 + 1" in Table 2). Some taxa have two, three, or even four rows of ovules on each flange. In the latter, the number of rows of ovules typically decreases to 1 + 1 apically, probably due to spatial constraints at the apex of the ovary. The number of ovule rows per carpel varied somewhat within a taxon and is correlated (as might be expected) with the degree of placental evagination (whether prominently or slightly 2-flanged). Because of this variation, we chose not to plot this feature on the cladogram (but see Table 2).

Only *Barberetta* of Haemodoraceae has a single ovule, correlated with a similar nutlet/achene fruit type. Several other taxa of Haemodoraceae (*Pyrrorhiza neblinae* Maguire & Wurdack and all species of *Dilatris* P. J. Bergius, *Phlebocarya* R. Br., and *Wachendorfia* Loefl. have one ovule per carpel, but these all occur within three functional carpels. All Philydraceae have numerous ovules per carpel, these with several rows of ovules per flange. *Haemodorum* has two rows of ovules per placenta and per carpel. *Tribonanthes* has multiple rows of ovules, but no placental flanges.

8. Septal Nectaries (Fig. 58, Table 2)

Pontederiaceae are variable with respect to the presence or absence of septal nectaries. Of twenty species investigated to date, seven species have septal nectaries: *Eichhornia crassipes, E. heterosperma, E. meyeri, E. paniculata, Pontederia cordata* var. *cordata, P. sagittata,* and *Reussia subovata* (see Fig. 1, 32; Table 2). Interestingly, *Eichhornia diversifolia* lacks septal nectaries, as do all investigated species of *Monochoria, Heteranthera* and the single species of *Hydrothrix* and *Scholleropsis.* These results corroborate those of the eight species observed by Strange et al. (2004); only the observation of lack of septal nectaries in *Eichhornia diversifolia* (which they did not observe) add to their general conclusions, indicating polymorphism in this likely polymorphic genus.

Of the seven taxa in Pontederiaceae that have septal nectaries, no significant variation was noted in structure. All occur within the tissues of the three septa and consist of one to two layers of densely staining epithelial cells (e.g., see Fig. 1, 5, 32). The position of the septal nectaries varies somewhat. All occur at or near the base of the superior ovary and extend from about 1/4 to over 3/4 the length of the ovary (Fig. 42). The species of *Pontederia* and *Reussia* examined have the longest relative septal nectary length, probably correlated with the fact that these taxa also have a single ovule with an apical placenta position.

Septal nectaries are found in the great majority of Haemodoraceae (see Simpson 1993) and are absent in Philydraceae (Table 2). A reconstruction of septal nectary gain/loss infers that presence of septal nectaries is an ancestral feature for Pontederiaceae, having been lost three times within the family: in the *Heteranthera/Hydrothrix* clade, in the *Monochoria* clade, and in *Eichhornia diversifolia* (Fig. 58). These independent losses may well be correlated with a shift in pollination mechanism, whereby a nectar reward is no longer adaptive.

CONCLUSIONS

These studies of the floral anatomy of the family Pontederiaceae reveal several characters that are generally well correlated with recent hypotheses of phylogenetic relationships (Graham et al. 1998). For example, tribe Pontederieae of Cook (1998), containing *Pontederia* and *Reussia,* seems well supported not only by a reduced functional carpel number (or "pseudomonomery," Fig. 51), reduced ovule number, and apical placental position (Fig. 56), which are features previously known from morphological studies and correlated with the anthocarp fruit type, but also in having a derived floral aerenchyma distribution (Fig. 52), lacking placental evagination (Fig. 57), and being the only Pontederiaceae with styloid or prismatic crystals. The clade containing *Monochoria, Pontederia,* and *Reussia,* and three *Eichhornia* species may be united by the presence of unique epithelial cells, although this would require a loss of such bodies in three *Eichhornia* species, which together form a distinct clade (Fig. 53). A clade containing the above taxa plus *Eichhornia crassipes* appears united in being the only taxa with com-

pletely synseptalous ovaries (Fig. 55). Heteranthereae of Cook (1998), equivalent to the *Heteranthera/Hydrothrix* clade appears united by the loss of septal nectaries, although septal nectaries were also lost in other members of the family, specifically in all *Monochoria* species and in *E. diversifolia* (Fig. 58). Some characters show a gradation of states, e.g., septal fusion.

This study also points out the need to further dissect some standard morphological characters, such as placentation. Our division of what is usually treated as a single character into three characters: septal fusion, ovule/placenta position, and placenta evagination, gives greater insight into the direction and significance of these floral features.

The adaptive significance of some observed floral anatomical features, such as crystal type and epithelial cell occurrence, is unknown, and others, such as placenta position and ovule number, are undoubtedly correlated with fruit type. However, the presence or absence of septal nectaries may be significant with respect to pollination mechanisms. Tannin cell occurrence and distribution might possibly be related to herbivory. The interesting occurrence of floral aerenchyma may be adaptive with respect to fruit floatation, a hypothesis that needs testing. An increased number of ovules and ovule rows may be an adaptation for increasing total seed output; the decreased number of ovules in *Pontederia* and *Reussia* is correlated with a specialized, single-seeded fruit type.

In conclusion, we feel that these floral anatomical data may be valuable in further elucidating the phylogenic relationships in Pontederiaceae. In particular, problems with rooting the tree (see Graham et al. 1998) may be solved with the addition of these non-molecular data to a molecular data set.

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